EXHIBIT 3

HUMAN GENE TRANSFER/THERAPY PROTOCOL

9007-002

Blaese, R. Michael, National Cancer Institute; *Treatment of Severe Combined Immune Deficiency (SCID) due to Adenosine Deaminase (ADA) Deficiency with CD34(+) Selected Autologous Hematopoietic Stem Cells*

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Title: Treatment of Severe Combined Immunodeficiency Disease (SCID) due to Adenosine Deaminase (ADA) Deficiency with Autologous Lymphocytes Transduced with a Human ADA Gene

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1.0 Introduction

1.1 Severe Combined Immunodeficiency (SCID)

SCID is a group of inherited immunodeficiency disorders characterized by profound cellular and humoral immune dysfunction resulting in recurrent opportunistic infections and failure to thrive with onset in the first few months of life (1). Those affected usually die within the first years of childhood unless successful immunologic reconstitution is achieved by transplantation. In 20-25% of SCID cases, a unique autosomal recessive form of the disorder can be identified which results in deficiency of the purine catabolic enzyme adenosine deaminase [ADA, EC 3.5.4.4] (2). ADA deaminates adenosine to inosine and deoxyadenosine (dAdo) to deoxyinosine (**Appendix 13.1, Figure 1**). In the absence of ADA, dAdo accumulates in the tissues and may be phosphorylated to form deoxyATP with accumulation of high intracellular levels of both (3-5).

The mechanisms through which these biochemical abnormalities result in combined immunodeficiency remain incompletely understood. The bulk of the evidence suggests that the intracellular accumulation of dAdo and its metabolites, particularly deoxyATP, results in inhibition of DNA synthesis and cell death(6-8). T-lymphocytes are preferentially affected in this disorder because they express higher concentrations of the kinases which phosphorylate deoxyadenosine to dATP than other tissues, resulting in T-lymphopenia and profound cellular immunodeficiency.

ADA deficiency with severe combined immunodeficiency is a genetically heterogeneous disorder with a variety of mutations having been identified in the ADA protein that are responsible for alterations in ADA activity and enzyme stability and survival (9-13). A spectrum of partial ADA enzyme deficiencies has been identified in a number of kindreds. These individuals have deficient erythrocyte ADA activity, 5-20% of normal ADA activity in their peripheral lymphocytes and are in general clinically healthy.

1.2 Bone Marrow Transplantation for the Correction of ADA Deficient Severe Combined Immunodeficiency

Bone marrow transplantation with HLA-matched marrow has been curative for ADA normal

[ADA(+)SCID] as well as ADA deficient [ADA(-)SCID] patients and is probably the treatment of choice for this disease (14-23). The overall success rate for bone marrow transplantation in SCID is >70%, but ADA(-)SCID responds less well than ADA-normal SCID (23). Pre-transplant cytoablation has usually not been required for HLA-matched marrow transplants for SCID because of the lack of host T-cell immunity which might otherwise reject the graft(24). Unfortunately, only about 30% of patients will have an HLA-matched sibling donor available and until recently transplantation from a mismatched donor resulted in severe and usually fatal graft versus host disease (GVHD). Evaluation of ADA(-)SCID recipients of HLA-matched or HLA-haploidentical transplants without cytoablation has demonstrated that some patients show persistence of donor cells only of the T cell lineage (24,26). Nevertheless, engraftment of donor T-cells (and/or their precursors) alone is sufficient to reconstitute dual system immune function in some of these SCID patients.

Alternative techniques for immune reconstitution of these SCID patients are being developed which in some cases may be nearly as successful as matched sibling donor transplantation. The development of methods to deplete mature T-cells from the donor marrow has substantially eliminated the complication of severe GVHD, previously the principal cause of death in HLA non-identical transplants (25).

In the absence of an HLA-identical sibling donor, T-cell depleted parental bone marrow is preferred over an unrelated donor in order to preserve some degree of histocompatibility between the engrafted immune system and the host. Such HLA-haploidentical or "half-matched" transplantation in SCID has been quite successful in ADA (+) SCID patients (26). However, a significant proportion of ADA(-) patients have failed to achieve stable engraftment when transplantation protocols lacking ablative conditioning were followed(17). Some of these cases have had to be prepared for transplantation by cytoreductive conditioning in order to achieve engraftment with T-cell depleted haploidentical marrow (18,19,27). The development of the B cell system, antibodies and specific immunity to immunization is delayed, sometimes for many months, in the recipients of HLA-haploidentical marrow in comparison with recipients of HLA-identical transplants.(28,29) In a recent presentation (Jeffrey Modell Foundation, April 19, 1990, in press), Richard O'Reilly, M.D., reported the results of T depleted haploidentical bone marrow transplantation in 32 patients with SCID. "Durable engraftment" was eventually achieved in 26 patients (81%). However, only 12 of the 26(46% or just 37.5% of all 32 patients treated with BMT) acheved functional B cell systems with this treatment.

The need for conditioning cytoreduction/immunosuppression with systemic chemotherapy and/or total body irradiation significantly increases the risks of both short and long term complications resulting from this treatment.(68) With busulfan/cyclophosphamide, the most widely used conditioning regimen, the patients routinely develop leukopenia and thrombocytopenia requiring blood and platelet transfusion support until engraftment provides sufficient endogenous production. During this period the patients are at significant risk hemorrhage and for the acquisition of bacterial, viral, and fungal infections and transfusion associated infectious agents. These treated children all develop alopecia and severe mucositis is a common problem, even in centers with considerable experience using this conditioning regimen. Cyclophosphamide can cause an acute cardiomyopathy which is frequently fatal. The first ADA(-)SCID patient we studied (and the donor of the TJF-2 cell line) died of cardiac necrosis/failure about 14 days after cyclophosphamide cytoreduction for a haploidentical bone marrow transplant. Busulfan is also known to cause cataracts and/or progressive pulmonary fibrosis in a small percentage of patients. Irreversible sterility is an almost universal consequence of these conditioning regimens. Other chemotherapeutic agents have a similar spectrum of significant acute and long term side effects as does total body irradiation (TBI). TBI may adversely affect the development of the CNS in young children. Secondary malignancies are also a complication of these treatments and have been seen in SCID transplant recipients who have not achieved complete reconstitution. In summary, bone marrow transplantation with cytoreduction is a treatment with lethal risk and a significant proportion of patients receiving haploidentical grafts have experienced less than complete immunologic reconstitution.

Not all SCID patients are candidates for bone marrow transplantation. There are children with ADA deficiency SCID who are too debilitated to tolerate cytoreductive therapy (18) as well as ADA deficient children who have a less severe immunodeficient phenotype for whom the risks associated with cytoreductive therapy are felt to be excessive (30,31). The overall treatment-related mortality of bone marrow transplantation with cytoreduction may be as high as 10-20% depending on the initial condition of the patient. Therefore, additional alternative treatments including the development of methods for immune augmentation, enzyme replacement by the infusion of ADA and enzyme replacement by insertion of a functioning human ADA gene into patient cells have all been vigorously explored.

1.3 Enzyme Replacement Therapy for the Treatment of ADA Deficient SCID

ADA deficiency is an attractive candidate for enzyme replacement therapy because deoxyadenosine, the precursor toxic substrate, is freely diffusible and therefore the detoxifying ADA enzyme may not have to enter all (or even any) of a patient's cells to be effective in lowering the intracellular deoxyadenosine concentration. ADA deficient children who were initially treated with repeated infusions of irradiated RBC from normal donors as a source of ADA have had transient clearing of toxic deoxyadenosine metabolites(32-39). While monthly transfusions have been clinically helpful for a few, most patients have experienced little or no response. This type of enzyme replacement therapy also has the associated increased risks of iron overload and transfusion associated infections including CMV, EBV, AIDS, and hepatitis.

There is no readily available source of large quantities of human ADA protein and, in any event, its very short serum half-life makes its use impractical for direct enzyme replacement. In an effort to prolong the functional half-life of infused ADA, bovine ADA was covalently attached to polyethylene glycol(PEG) (40). PEG functions to prolong survival and reduce immunogenicity *in vivo* (41-45). PEG-ADA is rapidly absorbed following intramuscular injection and has a plasma half-life of 48-72 hours. To date, reports of the treatment of 3 patients with PEG-ADA have been published, although 15 patients are currently being treated with this drug in the USA and Europe. The initial 2 patients reported by Hershfield *et al.* (40) had onset of recurrent infection in infancy and each had failed to engraft haploidentical bone marrow transplants. The 3rd patient reported by Levy *et al.* (31) had a later onset form (onset >2 years of age) with less severe immunodeficiency disease. Each child was treated with weekly intramuscular injections of PEG-ADA (15 U/Kg) to produce peak blood levels 2 to 3 times the normal whole blood ADA activity and trough levels of 1 to 1.5 times the normal blood ADA activity.

PEG-ADA injections at 15 U/kg reversed the primary biochemical abnormalities resulting from ADA deficiency with the level of red cell total dAdo nucleotides (dATP + dADP + dAMP) declining to less than 1 % of the pretreatment levels. In addition, PEG-ADA therapy reversed the deficiency of S-adenosylhomocysteine hydrolase (AdoHcyase), an enzyme which is irreversibly inactivated by dAdo (46-48).

The correction of these biochemical abnormalities was accompanied by the gradual development of increased numbers of T lymphocytes, although absolute T lymphopenia persisted in many cases. Function in the form of blastogenic lymphocyte responses to phytohemagglutinin (PHA)

also improved but was variable from time to time in a given patient. Total serum immunoglobulin levels also increased in those patients not already on IV gammaglobulin replacement therapy. These improved laboratory parameters have persisted with continued PEG-ADA injections and have been associated with an apparent decrease in the frequency of serious "opportunistic infections"; in addition, accelerated weight gain has been seen in some patients. However, evidence of total immune system reconstitution has not been seen. Many have not developed positive delayed type hypersensitivity skin reactions and several have been unable to produce specific antibodies to tetanus toxoid or other commonly tested antigens. Two children on PEG-ADA treatment have developed anti PEG-ADA neutralizing antibody which interfered with the treatment. Paradoxically this problem was reversed following administration of a course of immunosuppressive therapy to one immunodeficient child (70).

One of the ironies associated with the use of PEG-ADA in the treatment of ADA(-)SCID is that the modest increase in immune function achieved with this treatment suggests that a bone marrow transplantation from a haploidentical (ie., parental) donor without immunosuppression/cytoablation would fail because of graft rejection. Any transplant in this setting then would probably require full cytoreductive immunosuppressive conditioning with its attendant increased risk of complications or alternatively exposure to a period of withdrawal of PEG-ADA treatment to allow the condition of the immune system to deteriorate.

There are insufficient data to fully evaluate the long term value of PEG-ADA therapy, although its use does not provide a cure for this disease. It appears that in many cases, enzyme replacement converts severe combined immunodeficiency disease (SCID) to a disorder more closely resembling a partial combined immunodeficiency disorder (like the Wiskott-Aldrich syndrome). Without restoration of the capacity to develop a full range of normal specific immune responses, children with partial combined immunodeficiencies continue to be at increased risk of bacterial, fungal and viral infections, the development of autoimmune phenomena, and an increased risk of malignancy (49). Therefore, alternatives or additions to PEG-ADA therapy need to be developed.

Several alternatives to transplantation are under study in patients with ADA(+)SCID. In patients whose lymphocytes in culture demonstrate response to supplementation with IL-2, a trial of daily parenteral injections of IL2 has resulted in some evidence of clinical stabilization and enhanced immune function(50-52). Because of the inconvenience and discomfort of daily IL-2 injections, a new protocol using a longer acting and possibly less toxic PEG-IL2

preparation has been initiated (Carolyn Paradise, M.D., Cetus Corporation). The goal of these studies is to use infusion of the exogenous T cell growth factor to induce expansion of the patient's endogenous T cell population and therefore enhanse cellular immune function.

1.4 Growth of T Cell Lines from the Blood of Patients with ADA(-)SCID and Transduction with the hADA Gene Using Retroviral-Mediated Gene Transfer

When we began our studies of gene transfer for ADA deficiency we attempted to derive T cell lines from the blood of SCID patients in order to have a suitable *in vitro* system to assess the effectiveness of our gene insertion in correcting the primary biochemical consequences of the ADA deficiency. Because of the profound T lymphopenia in these patients, we were initially only successful in establishing lines when we used the transforming T lymphotropic virus HTLV-I to transform the rare T cells found in the blood of patient JF into an immortal cell line. We were successful in obtaining three T cell lines (TJF 1, 2, and 3) from this patient. The line TJF-2 was characterized in detail and had the phenotype of mature T cells (53). In brief, this line consisted of a mixture of CD4 and CD8 T cells that expressed the IL2 receptor and were dependent on IL2 for their growth. Independent clones from the parent line expressed a variety of different T cell receptor β chain gene rearrangement patterns indicating that they had a unique antigen specificity rather than all being clonally derived from the same differentiated precursor cell. Subsequently, we have established and characterized transformed T cell lines from 8 additional ADA deficient SCID patients (54) and in each case examined, the lines had the phenotype of mature T cells.

The initial experiments demonstrating correction of the biochemical defect in ADA deficient T-cells by the insertion of a functioning human ADA (hADA) gene were performed in these HTLV-1 immortalized T cells (53,55-57). We demonstrated that the murine retroviral vector, SAX, which contains an SV40 promoted hADA gene, could be stably inserted into ADA(-) SCID T cells in culture. These SAX-transduced T cells produced enzymatically active hADA at levels comparable to normal T lymphocytes in culture. The reconstituted T cells were shown to be 30-100 fold more resistant to the toxic effects of deoxyadenosine added to the culture medium than were the original ADA(-) T cells **(Appendix 13.1, Figure 2)**. Clones were prepared from the cultured ADA(-) T cell populations immediately after retroviral vector exposure to evaluate them for gene insertion and for receptor heterogeneity. Of the twenty-seven clones evaluated by Southern blot, 6 contained an inserted SAX vector (22% successful gene transfer)

and each of these SAX vector-containing clones produced hADA (**Appendix 13.1, Figure 3**). Thus the principal biochemical defect in ADA(-) SCID T-cells was efficiently corrected with retroviral-mediated gene transfer.

The ability to more readily recover T-lymphocytes from ADA deficient children on PEG-ADA treatment has allowed us to evaluate gene transfer and cell function *in vitro* without the necessity of prior cell transformation with HTLV-I. After testing a variety of culture conditions, stimulants and growth factors, we found that we were able to successfully grow large numbers of T cells *in vitro* from these patients. The optimal culture conditions defined to date includes AIM-5 medium supplemented with 50-1000 U/ml rIL2 and stimulated with the anti-T cell receptor monoclonal antibody OKT3. We have established non-transformed T cell lines from 5 patients and in 4 of these we have been able to expand these cells over 1000 fold in culture in a 3-5 week period.

We have tested a variety of vectors that carry the hADA cDNA driven by different promoters for their ability to express hADA in immortalized human lymphoid and myeloid cells (58). The promoters used include a human cytomegalovirus immediate early promoter (vector LNCA), the simian virus 40 (SV40) early promoter (vector LNSA), a promoter containing the lymphotropic papova virus enhancer (vector LNLA), the beta-globin promoter (vector LNBBA), and the Moloney murine leukemia virus promoter located in the retroviral LTR (vector LASN). The vector LNSA is similar in design to the SAX vector described above. Of all these vectors, the vector LASN directed the highest level of hADA expression when transferred into the DHL-9 human lymphoblastoid line (4 μM/hr/mg protein), a level at least comparable to ADA levels in normal lymphoid cells (0.3-0.5 μM/hr/mg)(9,12,59). The vector LASN contains the hADA [A] gene promoted by the 5' LTR [L] and a neomycin resistance [N] gene (Neo^R) promoted by an internal SV40 promoter [S]. When inserted into murine bone marrow cells, expression of hADA could be detected in recipient mice for 6 months after cell transfer (60). See **Appendix LASN.**

The hADA gene was inserted into exponentially growing non-transformed ADA(-) T cells by using the vectors that were the most active in human hematopoietic cell lines; SAX, LASN, LNSA, and LNCA. The latter three vectors were derived from the same vector construct as the LNL6 vector used in our N2/TIL human protocol. These vectors are packaged in the

amphotropic packaging line PA317 (61), which was the same packaging cell line used in the N2/TIL human protocol. These producer lines have remained helper virus free for several months of continuous culture. We have recently rederived a helper free SAX-producer line and during the course of the formal evaluation of this protocol we will continue to test this and other vectors to determine the most efficacious vector for clinical use. The Food and Drug Administration will evaluate the data and approve the vector ultimately selected for this protocol.

The LASN modified ADA(-) T cells were shown to express normal amounts of hADA, to express neomycin phosphotransferase, and to be resistant to the toxic effects of deoxyadenosine in culture. On average, the ADA(-)T cells were transduced at about the same frequency as we have achieved with the N2/TIL protocol using the LNL6 vector. The transduced cells were further evaluated for cell surface phenotype, helper cell activity for immunoglobulin production and for their intrinsic cytolytic characteristics. For example, these cells did retain some helper T cell function which augmented immunoglobulin production by normal B cells in culture. Importantly, the ADA gene-corrected patient T cells continued to proliferate in culture and survived much longer than the T cells which had not been corrected by ADA gene insertion. This data suggests that intracellular ADA may confer a survival advantage to ADA(-)T cells even in an environment lacking exogenous deoxyadenosine. See **Appendix 13.2, Figures 1-6.**

1.5 In Vivo Survival and Gene Expression of Retroviral Vector Modified T cells in Mice, Rhesus Monkeys and Man

We have demonstrated in mice, Rhesus monkeys and man that T-cells grown *in vitro* can be readily transduced with retroviral vectors and will survive and express the introduced genes for substantial time periods *in vivo* after reinjection. In the mouse, experiments with Sperm Whale Myoglobin-specific (SWM) T-helper cells have also demonstrated that IL2 expanded and gene transduced T-cells maintain their ability to function as T-helper cells for antibody production *in vivo.* In our experiments with human tumor infiltrating lymphocytes (TIL) transduced with the neoR gene, we have recovered vector containing T cells as long as 6 months after initial cell transfer and have observed no untoward effects of any kind associated with the transferred gene. Our colleague, Claudio Bordignon, has shown that PBL from ADA(-)SCID patients transduced with the hADA gene survive and function normally when transplanted into immunodeficient BNX mice while non-transduced ADA(-) PBL do not.(67)

1.5.1 In Vivo Experiments with the Murine T-helper cell clone 14.1 Transduced with the hADA Gene

These data were provided during the extensive review of the N2/TIL protocol approved January 19, 1989. In summary, these murine T cells were readily transduced with the SAX vector to express both neoR and hADA. Following ip injection of $25x10^6$ cells into nude mice, T cells expressing the hADA gene were found in direct spleen lysates and NeoR expressing cells could be recovered by culture and selection in G418 for up to 83 days after transplantation. The 14.1 cells are specific for the antigen SWM and the nude mouse recipients of these cells were able to produce antibody to SWM demonstrating the preservation of their intrinsic immune function when the cells were reinjected *in vivo*. Thus there was partial correction of immune function in these immunodeficient mice following transfer of IL2 expanded lymphocytes. See **Appendix 13.3, Figure 1.**

1.5.2 In Vivo Experiments with Rhesus T-lymphocytes Transduced with the N2 and SAX Vectors

T lymphocyte lines were established from adult Rhesus monkeys by stimulation of blood or lymph node cells with polyclonal activators followed by culture in rIL2 supplemented medium. Genes were introduced into these cells by retroviral-mediated gene transfer and the cells selected for expression of the NeoR gene in G418. Following infusion of from $5.4 x10^7$ to $1.4 x 10^8$ transduced T cells/kg, NeoR gene-expressing cells could be recovered from these animals as late as 158 days after transfer. A summary of these ongoing monkey studies is shown in **Appendix 13.3, Figure 2.**

1.5.3 In Vivo Experiments in Immunodeficient BNX Mice Injected with ADA(-)SCID Lymphocytes Transduced with the hADA Gene

Our collaborator Claudio Bordignon **(see attached letter)** has studied the function of ADA (-)SCID lymphocytes injected into immunodeficient mice carrying the bg/nu/xid (BNX) mutation. Freshly isolated ADA(-)PBL were stimulated with antigen or PHA and transduced with the hADA gene by multiple cycles of exposure to ADA vector supernatant. $20x10^6$ cells

were injected ip into BNX mice. 9 animals were reconstituted with this procedure, six with transduced cells and three with non-tranduced ADA(-)PBL as negative controls. Twenty BNX mice were reconstituted with normal PBL as positive controls for the efficiency of this human-mouse model. *In vivo* long term survival of human cells in the recipient BNX mice was demonstrated in the spleen by DNA analysis and the spleen lysates showed the typical band of human ADA by Cellogel analysis. Four weeks after reconstitution, human T and B cells were detected in the peripheral blood and spleen by FACS analysis.

Immune function of the transferred human cells was evaluated by testing for the presence of human IgG in the mouse blood and measurement of human alloreactive T cells in the spleens of the recipient BNX mice. The mice reconstituted with hADA transduced ADA(-)lymphocytes produced human IgG at levels comparable to BNX mice reconstituted with normal PBL. By contrast, no human IgG was detected in the blood of recipient mice reconstituted with the non-transduced ADA(-)lymphocytes, even though BNX mice have normal amounts of ADA in their own cells and do not have elevated levels of circulating or tissue deoxyadenosine. Allospecific human T cell clones were recovered from the spleens of BNX recipients reconstituted with transduced PBL, but not non-transduced PBL, and the recovered T cells expressed vector derived hADA. These observations suggest that intracellular correction of ADA(-)lymphocytes may be more beneficial for immune reconstitution than extracellular detoxification alone.

1.5.4 In Vivo Human Experiments with TIL Transduced with the LNL6 Vector

On January 19, 1989, Dr. James Wyngaarden approved our human gene transfer N2/TIL clinical protocol (protocol 86-C-183c). At present, 8 patients with advanced malignant melanoma have received tumor infiltrating lymphocytes (TIL) that have been marked with the safety-modified N2 vector called LNL6. The first 5 patients were treated between May 22 and July 21, 1989. Data from these first five patients has been prepared for publication and a preprint of the manuscript has been made available.

Because we realized that this initial human gene transfer clinical protocol would provide a basis for risk assessment for future human gene therapy protocols, we carried out extensive safety studies on: a) the retroviral supernatant prior to transduction of human TIL, b) the transduced TIL before they were infused into the patient, and c) the patients who received the gene-marked TIL.

The flowsheet demonstrating the studies carried out for each patient, a copy of the Certificate of Analysis - N2 Retroviral Supernatant, a copy of the Certificate of Analysis - Transduced TIL, and the flowsheet followed in growing the transduced human TIL are shown in **Appendix N2/TIL.** All the original data are provided to, and reviewed by, the FDA.

A summary of the safety data: 1) there have been no side effects or toxic reactions due to the gene transfer; 2) there has been no evidence of replication-competent virus in any retroviral supernatant used for clinical studies; 3) there have been no consistent differences in the pattern of cell growth, phenotype or cytotoxic function in any preparation of gene-transduced TIL; 4) no lymphocyte population has developed IL-2 independence; and 5) there has been no evidence of viral exposure for any of the 8 patients based on Western analysis as well as 3T3 amplification with S+L- of patient serum. In short, the data acquired to date (70 patient-months) have demonstrated no abnormalities, side effects, toxicities, or pathology due to the retroviral-mediated gene transfer procedure.

1.5.5 Assessment of the Safety of Retroviral-mediated Gene Transfer in Rhesus Monkeys and Humans

Nine monkeys that have received replication-competent murine amphotropic retrovirus have been followed for several years (a total of 32 monkey-years). Copies of the manuscript entitled "Amphotropic Murine Leukemia Retrovirus is Not an Acute Pathogen for Primates" have been made available to the Committee. At this time, there has been no pathology, malignancy, toxicity, or other abnormality that could be attributed to the retrovirus (not retroviral vector in this case but rather infectious virus) exposure except for transient retroviremia in one monkey. Retrovirus exposure is confirmed by the presence of an antibody response to the virus with ongoing positive Western analyses in several animals.

The conclusion from the monkey data is that, even though the long-term risk assessment is still to be determined, there is no detectable short-term pathology or illness caused by exposure of non-human primates to replication competent murine amphotropic retrovirus.

Since foreign DNA is inserted randomly into the genome of cells that are re-infused into the patient, there is some potential for the insertional event to result in an unfavorable outcome. If the insertion disrupts a gene essential for maintaining cell function, that particular cell might

die. More threatening is the possibility that insertion may initiate oncogenic transformation of the cell. The magnitude of the risk that gene transfer poses to a patient can not be accurately stated at present, but such a risk should be low. The data in primates accumulated so far indicates that the risk (at worse) should be considerably less than that associated with the conditioning cytoablation/immunosuppression regimens used for haploidentical bone marrow transplantation. In our 70 months of cumulative patient observation in the human gene transfer clinical protocol and the 32 years of cumulative observation of primates (some severely immunosuppressed) intentionally exposed to large amounts of infectious replication competent retrovirus, no untoward effects of retroviral exposure or retroviral-mediated gene transfer have been observed. The risk factors to consider are detailed in the review "Safety Factors Related to Retroviral Mediated Gene Transfer in Humans", copies of which have been made available to the Committee. Long-term surveillance of our exposed monkeys as well as the TIL patients will be necessary in order to obtain a better understanding of the actual frequency and severity of the risks associated with this procedure.

1.6 Summary and Rationale for Proposed Protocol

ADA(-)SCID is a profound dual system immunodeficiency disease (63). SCID is one of several genetic diseases which can be effectively treated by bone marrow transplantation (64). Children with SCID due to ADA deficiency can be cured by HLA-matched sibling donor bone marrow transplantation, probably the treatment of choice for this disease even though there can be morbidity and mortality associated with this procedure. Treatment for those children who do not have a suitable matched sibling donor is not as satisfactory. Alternative transplantation strategies as well as enzyme replacement techniques are being tested in this patient population. ADA(-)SCID patients may unfortunately not reconstitute with haploidentical marrow cells, may not be candidates for cytoablation due to antecedent lung or liver disease, or may have a milder phenotype that does not justify the risk of haploidentical bone marrow transplantation with preparative cytoablation. PEG-ADA therapy has resulted in some improvement in growth, a variable increase in the number of T-lymphocytes in the peripheral blood and a decrease in the incidence of severe infections.

Another approach to the treatment of severe genetic disease is the use of gene therapy (65,66). For the past 3 years, we have conducted experiments *in vitro* and *in vivo* that have documented that T-lymphocytes are suitable vehicles for gene transfer. The use of T-lymphocytes as gene therapy vehicles is especially significant in ADA deficient patients where the engraftment of T-

cells alone following bone marrow transplantation has resulted in recovery of both cellular and humoral immunity.

There are several pieces of evidence that suggest that infusions of ADA gene-corrected autologous T cells could be of therapeutic benefit for these patients:

1. Our experience with patients with metastatic cancer who have been treated with culture-expanded TIL has taught us that specific immune function (in this case anti-tumor immunity) can be greatly augmented by treating the patient with his own lymphocytes which have been expanded with IL2 *in vitro*. Therefore, *in vitro* expansion of cells may be more efficient than *in vivo* expansion in some cases.
2. Polyclonal expansion of autologous T cell numbers *in vivo* by injections of IL2 has resulted in some therapeutic benefit in ADA(+)SCID patients. Polyclonal expansion *in vitro* could be expected to also be of benefit.
3. The generally weak antigen specific responses seen in PEG-ADA treated patients suggests that these patients continue to have difficulty in maintaining clonal expansion of their antigen-primed lymphocyte populations *in vivo*. This may be a consequence of deficient intracellular concentrations of ADA.
4. The *in vitro* survival of ADA(-) T cell lines is markedly enhanced if they have been genetically corrected by insertion of a functional ADA gene even though these cells are not exposed to elevated 2'deoxyadenosine concentrations in the extracellular medium.
5. The observation that freshly obtained ADA(-)PBL transduced to express the hADA gene had a significant survival and functional advantage over non-transduced ADA(-) lymphocytes when transplantated into immunodeficient (but ADA normal) BNX mice strongly suggests that intracellular ADA provides significantly more reconstitution to the cells of the immune system of these patients than does extracellular enzyme alone.

These observations suggest that patients treated with their own hADA gene transduced lymphocytes could experience immune augmentation which would surpass the partial immunologic reconstitution seen with PEG-ADA therapy alone. In addition, the ADA contained in the cells returned to the patient could serve as a cell-associated repository of ADA enzyme capable of detoxifying deoxyadenosine diffusing from extracellular sites. These observations serve as the rationale for the present clinical proposal.

We therefore propose a two part clinical trial. Part 1 would study the effects of repeated

infusions of autologous lymphocytes polyclonally stimulated in culture to permit retroviral-mediated transduction with a vector containing the hADA gene as well as the selectable gene Neo^R (vector LASN). Selection for gene-expressing cells will not be performed during Part 1 in order to shorten the culture period to maximize polyclonality of the reinfused ADA gene-corrected cells. No change to current PEG-ADA or immunoglobulin replacement therapy would be instituted during **either part** of this protocol. Information derived from Part 1 will provide data about the therapeutic effects of the ADA reconstituted cells on patient immune function as well as possible toxicities associated with cellular immunotherapy, survival of the transduced cells *in vivo*, and continued expression of the introduced genes *in vivo*.

Part 2 of the proposal will consist of infusions of transduced cells which have been selected for expression of the introduced genes. During part 2.A. of the protocol, the autologous T cells will be infused as soon as they reach a level of hADA expression during culture selection which is similar to the ADA concentration found in normal blood cells. In part 2.B., an escalating schedule of infusions of the selected ADA gene-transduced cells will be given to determine whether sufficient cells could be given to eventually replace the requirement for PEG-ADA treatment. Based on the average level of ADA expression in ADA deficient T cells transduced with the LASN vector this calculated cell number is approximately $1x10^9$/Kg. This cell dosage is similar to the number of cells infused during TIL therapy for cancer.

In our original protocol submission we included a Part 3 during which PEG-ADA treatment would be stopped. This portion of the protocol has been withdrawn from this version because its design is critically dependent upon data to be generated during Parts 1 and 2 of the protocol, data we cannot predict with sufficient accuracy in advance of the actual human studies. We will return to the committee with an amended protocol containing the data from parts 1 and 2 if we determine that part 3 is indicated for certain patients.

2.0 Objectives

A) To evaluate the possible therapeutic efficacy of the administration of autologous lymphocytes transduced with a normal hADA gene in an effort to reconstitute the function of the cellular and humoral immune systems in patients with ADA(-)SCID.

B) To evaluate the *in vivo* survival of culture-expanded autologous T cells and the duration of expression of the inserted genes.

3.0 Selection of Patients

Children with documented SCID secondary to ADA deficiency. All patients to be treated by gene transfer under this protocol will be concurrently treated with PEG-ADA unless they have become refractory or allergic to this drug. The decision to treat with PEG-ADA rather than bone marrow transplantation will have been made by the patient's parents and the child's physician independent of input by the NIH investigators and prior to consideration for enrollment in this protocol. Those patients receiving PEG-ADA will have been treated with at least 15 U/kg for a minimum of 9 months before enrollment. This is to permit evaluation of the extent of immune reconstitution achieved with PEG-ADA alone. To be eligible for enrollment, the initial patients must have objective evidence of incomplete reconstitution despite PEG-ADA treatment. These objective measures are listed below. Persistent or recurrent thrush will also be considered evidence of deficient T cell function.

Chronic pulmonary and gastrointestinal infections are common in these patients. Children with significant pulmonary disability defined as hypoxia at rest (<91% O2 saturation) will not be enrolled in part 2.B. of this protocol until sufficient experience has been obtained with lymphocyte infusions in children with ADA(-) SCID to be comfortable that the infusion of large numbers of cells will not expose the patient to unacceptable risk. Children with acute hepatitis or those with chronic liver disease resulting in significantly impaired liver function will also be deferred from Part 2.B. until the tolerance of this procedure is established in children with more normal hepatic function. Treatment of children currently experiencing acute infections will be postponed until satisfactory infection control has been accomplished. HIV screening will be performed by the referring physician and children with HIV infection will not be accepted for this study. Confirmatory non-serologic studies for HIV status will be performed at the NIH. Children with HIV infection will be excluded. Patients with malignancy will not be accepted.

Immunologic Inclusion Criteria: the patients must manifest any three of the following immunologic features to the eligible for inclusion in this protocol.

1. Absolute lymphopenia, (<1500/mm^3)
2. T-cell lymphopenia, (<1000/mm^3)
3. CMI skin test panel (7 antigens + 1 control), < 3 of 7 positive
4. *In vitro* antigen stimulated lymphocyte proliferation, < 30% of control for 2 or more antigens
5. *In vitro* proliferation to allogeneic cells, < 50% of control
6. *In vitro* proliferation to mitogens, < 50% of control
7. *In vitro* T helper function, < 50% of control
8. *In vitro* T-cell cytotoxicity, < 50% of control against allogeneic targets or virus-modified autologous targets
9. Deficient (< 40% of control) immunoglobulin production *in vitro*
10. Deficiency of serum IgG, IgA, or IgM, (more than 2 S.D. below normal mean)
11. Isohemagglutinins, titer of < 1:16 by age 3
12. Deficient specific antibody responses to challenge with non-viable vaccine antigens based on norms for the response to each. Responses to the bacteriophage antigen øX174 have been well characterized by Ochs, et al. Our own laboratory has established norms for responses to pneumococcal polysaccharides types 1, 2, and 3 as well as to KLH and Brucella abortus antigens. Norms are currently being established for responses to VEE and RVF vaccines.

Patients admitted for study under this protocol will be registered with the PI at Building 10, Room 6B05, National Institutes of Health, Bethesda, MD. 20892. [(301) 496-5396]. The PI is also to be notified immediately whenever a protocol patient is taken off study.

4.0 Clinical Evaluation Prior to Treatment

Prior to enrollment into this protocol the child will be admitted to Patient Care Unit 3 B South of the Clinical Center for an evaluation to determine eligibility as per Metabolism Branch Protocol 66-C-77, which permits the immunologic evaluation outlined in section 4.2.

4.1. Obtain and review previous records detailing clinical history, physical examination, general laboratory and specific immunologic evaluations. Complete history and physicial examination at admission. Radiographic evaluation as indicated, depending on availability of

films from outside sources.

4.2 Complete pretreatment immunologic evaluation to include:

a. Freeze storage of 4 ml serum for future measurement of antibody levels. Antigens to be tested can include Hepatitis B, HIV, Tetanus, Diphtheria, Pneumococcal polysaccharide types 1, 2, and 3, *Hemophilus influenzae*, Keyhole limpet hemocyanin (KLH), ØX174, Riff Valley Fever (RVF) virus, and Venezuelan equine encephalitis (VEE) virus. The final panel to be measured will be at the discretion of PI depending upon the clinical situation, history of previous immunizations and gammaglobulin replacement therapy, and antigens used in the evaluation of patient response to treatment with gene transduced lymphocytes.

b. Isohemagglutinins and quantitative immunoglobulins (A,G,M and E)

c. Cellular phenotype of peripheral blood by dual label FACS analysis (CD3/HLA-DR, CD4/CD8, CD45/CD14, CD5/CD20, CD2/CD16)

d. *In vitro* lymphocyte proliferative responses to mitogens (PHA, PWM, Con-A), soluble antigens (Diphtheria, Tetanus, Candida albicans), alloantigen and anti-CD3. *In vitro* analysis of B cell responsiveness, helper and suppressor T cell function will also be performed.

e. Measurement of ADA concentration in peripheral mononuclear cells and concentrations of deoxyadenosine in red cells, serum, and urine, and dAXP in RBC and blood mononuclear cells as available and appropriate.

f. Determination of cytotoxic cell function (NK, LAK, CML and virus modified self target killing, as avaliable)

g. DTH skin test panel (CMI Multitest or equivalent)

4.3 Routine Laboratory Tests.

a. Chemistry panel to include: Uric acid, Ca, Phos, SGOT, SGPT, Alk. P'tase, LDH, bilirubin, BUN, creatinine, total protein, glucose.

b. CBC, differential and platelets

c. Urinalysis

d. Non-serologic HIV test

4.4 Review of PEG-ADA treatment diary of growth, illnesses, etc. if available.

5.0 Stratification and Randomization

Not applicable

6.0 Nature of Procedures or Therapeutic agents

6.1 Isolation and Culture of Peripheral Blood Mononuclear Cells from the Patient

The child will be admitted to the Clinical Center of the National Institutes of Health. A phlebotomy or lymphopheresis (performed by the NIH Apheresis Unit according to age-appropriate procedures following the previously approved Metabolism Branch Protocol 82-C-44) will be used to obtain lymphocytes. 7 ml of blood/Kg will be sampled per leukaphoresis and a maximum of 7 ml/kg will be obtained by phlebotomy every 4 weeks. Fresh peripheral blood mononuclear cells (MNC's) will be separated from the red cells and neutrophils by Ficoll-Hypaque density gradient centrifugation. The MNC's will then be washed, counted and cultured at approximately $1x10^6$ cells/well in 24 well tissue culture plates in AIM-V* which consists of AIM-V (GIBCO) with 2 mM glutamine, 50 U/ml penicillin, 50 µg/ml streptomycin, 2.5 µg/ml Fungizone and 25-1000 units/ml of IL-2 (Cetus). At the initial plating,10 ng/ml OKT3 (Ortho) monoclonal antibody will be added to each well. The cells will be cultured at 37°C in a humidified incubator with 5% CO_2. The conditions of culture and lymphocyte stimulation may be modified by the PI during the course of this protocol to take advantage of improvements in technique or media.

6.2 Growth, Transduction and Selection of ADA-Deficient T-Lymphocytes

Once the T-lymphocytes have begun to proliferate (usually 24-96 hours after initiation of the culture), LASN vector-containing supernatant (containing protamine 5-10 µg/ml and up to 1000 U/ml IL2) will be added to the wells after aspirating off the top half of the medium. This will be repeated 1-2 times daily for a period of up to 7 days. After the final exposure to retroviral vector, the cells will be fed with fresh AIM-V* and cultured another 2 to 7 days to permit the cultures to return to exponential growth. In part 1, approximately 80% of the culture will then be infused into the patient and the remaining cells returned to culture for continued growth and selection procedures, and/or analyzed for phenotype, T cell repertoire, and percentage of cells demonstrating vector integration. Selected cultures will be periodically

analyzed for the above features and cryopreserved for future patient infusion.

In part 2.A., following transduction the cells will be allowed to regain exponential growth and then will be placed under selection with 0.15-1.0 mg/ml G418 and/or 200-1200 μM 2'deoxyadenosine for 1 to 14 days. The optimal conditions for selection will have to be established for each patient. The cells will be selected until the ADA level in the cultured cells reaches at least 80% of the level of ADA in normal blood MNC. At this point, approximately 80% will be infused into the patient and the remainder will be returned to culture and expanded in number for possible future use in part 2.B. The cells will then be subcultured every 2-8 days as needed and transferred into 6 well plates, flasks, gas permeable bags and/or a hollow fiber culture system as the cells expand in number by techniques similar to those used in the previously approved N2/TIL protocol 86-C-183. Again, conditions may be modified by the PI to take advantage of technical advances or to optimize results for an individual patient.

In part 2.B. of the protocol, the ADA gene-transduced, selected T cell population will be selected to enrich for cells expressing the introduced genes as described above and will be expanded in number so that as many as $3x10^9$/kg are available for infusion. Mixtures prepared from cryopreserved aliquots of earlier cell cultures may be made to broaden the infused repertoire should the cultures appear to have become oligoclonal based on the pattern of TCR_β chain gene rearrangements in the cultured populations.

An aliquot of the cells infused into the patient will be saved and we will subsequently perform Southern analysis on the DNA from the cultured cells after digestion with a restriction endonuclease which does not cut within the vector sequence to determine whether the gene-modified cells are polyclonal with respect to retroviral insertion. We will also probe for T cell receptor β chain gene rearrangements to address clonality with respect to T cell specificity as we have done in the N2/TIL protocol. (69) Cells will also be tested for their responses to IL-2 withdrawal and for replication competent retrovirus by S+L- with 3T3 amplification.

6.3 Reinfusion of hADA Transduced T-Lymphocytes

The transduced cells will be harvested, washed and resuspended in normal saline. The final cell preparation will be filtered through a platelet filter and transferred into a syringe or transfusion pack for infusion. A test dose of 2-5% of the total volume will be infused by

peripheral vein followed by an observation period of 5-10 minutes.

The initial infusion will not exceed $6x10^7$ cells/kg body weight. Total volume of infused cells will not exceed 10 ml/kg of body weight per day and the infusion should usually be completed within 120 minutes. The cell suspension will be mixed gently approximately every 5 minutes during the infusion while the child is being monitored for acute and subacute side effects. Antipyretics, antihistamines, narcotics and/or anti-inflammatory agents will be administered based on the experience gained in the N2/TIL protocol 86-C-183 as needed to control symptoms associated with the infusion. Substantial or persistent symptoms or signs of toxicity may require discontinuation of the infusion or consideration of intraperitoneal infusion of the cells (see section 7.1). Once the optimal conditions for infusion have been identified, all subsequent infusions will be based upon this experience.

6.4 Pharmaceutical Information

PEG-ADA. Each patient on this protocol will be concurrently treated with PEG-ADA unless there is clear evidence that they have failed treatment with this agent. PEG-ADA (Adagen) is commercially available and is supplied as a sterile liquid for injection manufactured by ENZON Inc., South Plainfield, N.J. It is administered as an intramuscular injection of 15-30 units/kg once or twice weekly. Each patient will have been treated with this drug for a minimum of 9 months before enrollment on this protocol and will thereafter be maintained on the previously determined optimum dosing schedule. The drug is supplied in a form ready for injection. There have been no reported toxicities or side-effects associated with its use at the recommended dosages.

7.0 Dose of Therapeutic Modifications

7.1 Part 1: Infusion of hADA Gene Transduced, Non-selected Lymphocytes.

In part 1 of this protocol we plan to administer the corrected T cells as soon as possible after gene introduction in culture. This is designed to minimize the potential for clonal overgrowth in culture and therefore the development of a disproportionate mix of immune specificities that might be significantly different from those present in the fresh blood sample. Experiments in immunodeficient mice transplanted with human cells (including gene treated ADA(-)SCID

The potential discomforts and risks in part 2 are the same as those in part 1. Repeated blood sampling and gene insertion could result in the repeated exposure of a single lymphocyte to additional gene insertion events. Part 2.B. will end after the patient has received approximately 6 infusions of autologous T cells at a dose/infusion calculated to be roughly equivalent to the weekly dose of PEG-ADA (15 U/kg). Four weeks after the last infusion, a thorough immunologic evaluation will be performed as will an estimate of the persistence of the gene-modified cells in the patient's blood. If no evidence of immunologic benefit from parts 1 and 2 are shown and if less that 1% of the blood lymphocytes represent persisting infused cells, the intravenous infusions of the patient will cease. The patient will then be treated with a modified protocol using intraperitoneal cell injections for approximately 6 infusions at a cell dose of $2\text{-}5x10^9$/kg. With clear evidence of immunologic benefit, periodic infusions of ADA gene-modified autologous T cells will be continued indefinitely. The benefits of part 2 could include significant immunologic improvement for the patient. We should also learn important information concerning the effects of repeated infusions (as opposed to single infusions) of substantial numbers of autologous ADA-corrected T cells on the survival of those T cells *in vivo*. Part 2 should last from 12 to 18 months and will require about 12 patient visits to the Clinical Center.

If this gene correction protocol does not prove to be superior to PEG-ADA alone, the protocol will be retired. If this treatment protocol proves to be superior to PEG-ADA alone, we will continue to indefinitely offer periodic infusions of ADA gene-modified T cells along with PEG-ADA treatment.

The NIH will provide the PEG-ADA and any IVIG required during the period of participation in this study and will continue to provide these drugs after completion of the protocol in order to maintain close follow-up of each patient indefinitely. If the therapy proves to be beneficial, we will continue to offer infusions of ADA gene-modified lymphocytes indefinitely.

8.0 Study Parameters

8.1 Evaluations Following Each Cell Infusion

Eighteen to 72 hours after completion of each treatment blood will be obtained for:

a. CBC, differential, platelets

b. Chemistry panel
c. T and B cell phenotype (as in 4.2.c)
d. Lymphocyte proliferative responses (as in 4.2.d)
e. Cellular ADA concentration
f. Analysis for vector DNA, such as by semiquantitative PCR or Southern analysis.
g. Bank serum for future analysis of Ig levels, antibody titers, etc.

8.2 Evaluations at the End of Part 1, Part 2.A. and Part 2.B.

a. The tests listed under 8.1 above
b. Skin test panel for DTH
c. Immunoglobulin levels and isohemagglutinin titers
d. Functional evaluation of B cell responsiveness and helper and suppressor T cell activity.
e. Evaluations of NK, LAK, CML and anti-viral cytotoxic activity in PBL, as available.
f. Immunization of the patient with an antigen such as Tetanus toxoid, Diphtheria toxoid, KLH, pneumovax, H. Influenza polysaccharide, Brucella abortus, øX174, VEE or RVF; obtain weekly blood samples x4 for antibody titers
g. PCR of blood lymphocytes for retroviral envelope
h. Western blot analysis of serum for antibody to retroviral antigens

8.3 Additional laboratory analyses during the course of treatment.

a. Between the third and fourth cell infusions of each Part, the patients will be immunized with a non-viable vaccine antigen to prime the peripheral lymphocyte pool obtained at the next phlebotomy to this new antigen. Following infusion of ADA gene-modified lymphocytes obtained after this immunization, we will carefully monitor the peripheral lymphocytes for antigen specific reactivity to this antigen including analysis of antigen induced proliferation, cytokine production, cytotoxicity, and antibody production as available.
b. Studies of lymphocyte functional capacity including attempts to isolate alloantigen and neoantigen reactive clones and DTH to the neoantigen if available.

8.4 Yearly follow-up laboratory evaluation.

a. CBC with Differential count
b. Immunologic evaluation to include:
   a) Serum immunoglobulins
   b) DTH skin test panel
   c) Isohemagglutinin titer
c. PCR on mononuclear cell DNA for vector sequences
d. Western analysis of serum for antibody to retroviral antigens
e. If at any time a malignancy develops, involved tissue to be analyzed for vector DNA

It is understood that the performance of an individual study or test as specified in this protocol is subject to factors such as patient compliance, scheduling difficulties, equipment malfunction, or the clinical judgment of the principal investigator or patient care physician, and that a test therefore may not be done in an individual instance with no violation of the protocol. However, any systematic modification of the original protocol in this regard, whether related to patient safety or not, will be submitted to the IRB for approval.

9.0 Off-Study Criteria

The development of any grade 3 or 4 toxicity that is not easily correctable. The toxicity sheet for this study (similar to the one used in the approved protocol 86-C-183 except for age/weight appropriate pediatric modifications) is included in the **Appendix-Common Toxicity Criteria.** This protocol will be terminated if two (2) treatment related deaths occur.

10.0 Evaluation of Results

10.1 Criteria for Response

Laboratory or clinical evidence of improved immune function such as development of more than one new positive delayed type hypersensitivity skin test to environmental antigens or immunogens, the development of antigen specific proliferative responses *in vitro* to antigens were no responsiveness existed prior to therapy, the production of specific antibodies to

antigenic challenge when the patient was previously unresponsive, the development of normal lymphocyte counts in a previously lymphopenic patient, or the resolution of a chronic infection not being treated with specific antimicrobial therapy.

10.2 Duration of the Study

Part 1 of this protocol is expected to last 6-9 months. Part 2.A. is expected to require an additional 6-9 months. The duration of part 2.B. is dependent on the response of the individual patient but should last at least 6 months. Children who have demonstrated clear evidence of immunologic improvement will be maintained on periodic ADA gene-modified lymphocyte infusions indefinitely.

11.0 Schema

Part 1.

Leukapheresis or phlebotomy to obtain peripheral blood lymphocytes.
↓
Induce lymphocyte proliferation in culture with anti-CD3 and IL2
↓
Transduce proliferating lymphocytes with LASN vector
↓
Resuspend lymphocytes in fresh medium and grow for 2 to 7 days
↓
Infuse approximately 80% of culture into patient. Analyze aliquot of the remaining cells as well as establish culture under selection for gene expression. Analyze and cryopreserve selected cells.
↓
Repeat procedure monthly for approximately 6 treatments
↓
Comprehensive immunologic evaluation

Part 2.A. ↓
Repeat above procedure with infusions of selected cells. The selected cells can be prepared from fresh blood samples or previously characterized cryopreserved cells can be given.
↓
Repeat infusions of selected cells for approximately 6 treatments
↓
Comprehensive immunologic evaluation

Part 2.B. ↓

Repeat above procedure for the growth and characterization of transduced/selected

↓ lymphocytes.

Infuse 10^8 transduced and selected cells/kg.

↓ approx. 4 weeks

Infuse $3x10^8$/kg.

↓ approx. 4 weeks

Infuse $1x10^9$/kg.

↓ approx. 4 weeks

Infuse $2\text{-}3x10^9$/kg.

↓

Repeat infusions at this level for up to 6 treatments at monthly intervals.

↓

Comprehensive immune evaluation.

↓

Continue periodic infusions if patient response is positive or begin course of 6 approximately monthly ip infusions of $2\text{-}5x10^9$ transduced/selected cells/kg.

↓

Repeat comprehensive immune evaluation

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13.0 Appendix

Appendix 13.1

Figure 1. Purine catabolic pathway demonstrating site of action of ADA.

Figure 2. The effect of ADA gene insertion on the inhibitory effect of 2'deoxyadenosine on the proliferation of normal and ADA(-)SCID T cells [TJF-2 cells] in vitro.

Figure 3. ADA concentration in TJF-2 clones prepared immediately after transduction with the SAX vector.

Appendix LASN.

Specific data regarding characterization of the LASN retroviral vector.

Appendix 13.2

Figures 1-6. Characteristics of ADA(-) T cells transduced with the LASN vector.

Appendix 13.3

Figure 1. Sperm whale myoglobin specific antibody responses of nude mice treated with human ADA gene-transduced murine T cells which had been maintained in culture for several months.

Figure 2. Current status of studies of Rhesus monkeys infused with autologous T lymphocytes which have been transduced with the NeoR and/or hADA genes using retroviral vectors.

Letter: from Dr. Claudio Bordignon.

Appendix N2/TIL. Flowsheets and Certificates of Analysis used in the N2/TIL protocol as an illustration of the kind of record keeping and variety of testing to be performed on this protocol.

Common Toxicity Criteria. Cancer clinical trials common toxicity criteria for pediatric patients.

Informed Consent Documents.